Exercise training-induced effects on the abdominal subcutaneous adipose tissue phenotype in humans with obesity

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RESEARCH ARTICLE

Exercise training-induced effects on the abdominal subcutaneous adipose tissue phenotype in humans with obesity

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Stinkens R, Brouwers B, Jocken JW, Blaak EE, Teunissen-Beekman KF, Hesselink MK, van Baak MA, Schrauwen P, Goossens GH. Exercise training-induced effects on the abdominal subcutaneous adipose tissue phenotype in humans with obesity. *J Appl Physiol* 125: 1585–1593, 2018. First published September 13, 2018; doi:10.1152/japplphysiol.00496.2018.—Rodent studies have indicated that physical exercise may improve adipose tissue function. We investigated the effects of a 12-wk supervised, progressive exercise training program on adipocyte morphology and abdominal subcutaneous adipose tissue function in metabolically well-phenotyped subjects with obesity. Men with obesity ($n = 21$) participated in a 12-wk supervised, progressive, combined exercise training program consisting of aerobic exercise (30 min at 70% of maximal power output 2 times/wk) and resistance exercise (3 × 10 repetitions at 60% of 1 repeated maximum 1 time/wk), with adjustment of exercise intensity every 4 wk. At baseline and after intervention, abdominal subcutaneous adipose tissue biopsies were collected to determine *1*) adipocyte morphology, *2*) gene expression of markers for lipolysis, inflammation, browning, adipokines, and mitochondrial biogenesis/function, *3*) protein expression of mitochondrial oxidative phosphorylation (OXPHOS) complexes, and *4*) ex vivo basal and β_2-adrenergic stimulated lipolysis. The exercise training program, which increased maximal aerobic capacity ($P < 0.001$) and muscle strength ($P < 0.001$), slightly reduced adipose tissue mass (~0.7 kg, $P = 0.021$) but did not affect abdominal subcutaneous adipocyte size ($P = 0.744$), adipose tissue gene expression of markers for mitochondrial biogenesis and function, browning, lipolysis, inflammation and adipokines, total OXPHOS protein content ($P = 0.789$), or β_2-adrenergic sensitivity of lipolysis ($P = 0.555$). A 12-wk supervised, progressive exercise training program did not alter abdominal subcutaneous adipocyte morphology and adipose tissue gene/protein expression of markers related to adipose tissue function or β_2-adrenergic sensitivity of lipolysis in male subjects with obesity.

NEW & NOTEWORTHY Studies that investigated the effects of exercise training on adipose tissue function in well-phenotyped humans are scarce. We demonstrate that 12 wk of supervised exercise training improved physical fitness and peripheral insulin sensitivity but did not alter abdominal subcutaneous adipocyte morphology, adipose tissue gene and protein expression of markers related to adipose tissue function, or β_2-adrenergic receptor-mediated lipolysis in men with obesity. A prolonged and/or more intense training program may be required to improve human adipose tissue function.

abdominal subcutaneous adipose tissue; exercise training; gene expression; obesity; protein expression

INTRODUCTION

The obesity epidemic is paralleled by a tremendous increase in the prevalence of obesity-related diseases, including type 2 diabetes (T2DM), nonalcoholic fatty liver, cardiovascular disease, and certain types of cancer (23). A sedentary lifestyle is a major contributor to obesity and related complications. In line, increased habitual physical activity and exercise training may have beneficial effects on insulin sensitivity and glucose homeostasis in patients who are obese, insulin resistant, and have T2DM (27, 28, 36). Therefore, increasing physical activity is a recommended lifestyle modification in the prevention and treatment of obesity-related disorders, including T2DM (10).

Since skeletal muscle is responsible for the majority of glucose disposal, adaptations in skeletal muscle metabolism are thought to play a central role in the exercise training-induced improvement of insulin sensitivity. Adipose tissue dysfunction in obesity, however, represents a key step in the development of obesity-related insulin resistance and chronic diseases (16, 38). The reason for this is that adipocyte hypertrophy in obesity promotes low-grade inflammation and decreases the adipose tissue lipid buffering capacity. Consequently, lipids accumulate in nonadipose tissues (e.g., skeletal muscle and liver) when lipid supply exceeds fat oxidation, thereby accelerating the development and progression of insulin resistance and chronic metabolic diseases (16, 38, 47).

Interestingly, there is evidence that exercise training may improve white adipose tissue function (45). Several rodent studies demonstrated that exercise training increased adipose tissue mitochondrial biogenesis (48, 51) and function (44, 46, 53), induced browning of white adipose tissue (5, 9, 46, 48, 51), and altered adipokine expression (6, 56). Furthermore, transplantation of white adipose tissue from trained animals to untrained recipients markedly improved skeletal muscle glucose uptake (46), suggesting that the improvement of adipose

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tissue function may contribute to the increased peripheral insulin sensitivity after exercise training. However, human studies that investigate the effects of exercise training on the adipose tissue phenotype are scarce. Exercise training has been shown to increase human adipose tissue gene expression of peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α) (39) and oxidative metabolism markers (37), yet conflicting data regarding the expression of adipokines and markers of lipolysis in human adipose tissue have been reported (3, 11, 20, 22, 29, 32, 35, 42, 50). Furthermore, the evidence that exercise training enhances adipose tissue lipolysis, assessed either ex vivo in isolated adipocytes or in vivo at rest and in response to a lipolytic stimulus, is inconsistent and complicated by confounding factors such as recent energy balance, as reviewed elsewhere (49). Importantly, most human studies that have investigated the exercise training-induced effects on adipose tissue metabolism did not perform detailed metabolic phenotyping, and metabolic status among other endogenous factors may determine the impact of an exercise training intervention on study outcomes (43).

The aim of the present study was to investigate the effects of a 12-wk supervised, progressive, combined exercise training program on abdominal subcutaneous adipocyte morphology, adipose tissue gene expression of markers related to mitochondrial biogenesis/function, browning, lipolysis, inflammation, and adipokine and protein expression of mitochondrial oxidative phosphorylation (OXPHOS) in individuals with obesity. Furthermore, using isolated adipocytes from these subjects, we determined the exercise training-induced effects on ex vivo basal and β_2-adrenergic stimulation of lipolysis. We hypothesized that this exercise training program induces beneficial changes in adipocyte morphology and gene and protein expression, as well as ex vivo basal and β_2-adrenergic stimulated lipolysis.

METHODS

Study participants. Sedentary middle-aged (40–70 yr) men who were overweight/obese ($n = 21$; including 7 T2DM patients) participated in the present study, which was conducted within the framework of a larger clinical trial designed to primarily investigate the effects of exercise training on liver fat content as well as hepatic, adipose tissue, and peripheral insulin sensitivity in individuals with nonalcoholic fatty liver and body mass index-matched controls (NCT01317576) (7). At screening, patients with T2DM were allowed to be on sulphonyl urea, metformin, dipeptidyl peptidase-4 inhibitors therapy (or a combination) for at least 6 mo with stable dosage for at least 2 mo or on a dietary treatment for 6 mo with fasting plasma glucose concentrations ≥7.0 and <10.0 mmol/l. All subjects gave written informed consent before participation in the study. The Medical Ethical Committee of Maastricht University Medical Center$^+$ approved the study protocol, which was performed according the principles expressed in the Declaration of Helsinki.

Study design. All participants were asked not to change their habitual dietary intake during the study period. General exclusion criteria were unstable body weight, cardiovascular disease, impaired renal function, hemoglobin <7.5 mmol/l, blood pressure >160/100 mmHg, participation in a weight loss or exercise program, history of substantial alcohol use (>3 units/day), history of drug abuse, use of beta-blockers, antithrombotic medication, insulin therapy, and use of medication known to interfere with glucose homeostasis (except for the patients with T2DM).

At screening, routine laboratory analyses and physical examinations were performed, medical history was checked, and a resting electrocardiogram was taken. Maximal power output (W_{max}) and maximal aerobic capacity ($\dot{V}O_{2max}$) were assessed during a graded cycling test with concurrent electrocardiogram until exhaustion. Body composition was determined using DEXA (Hologic Discovery A, Waltham, MA). Furthermore, 4 days before the start of the exercise training protocol and 48–72 h after the last exercise bout that was part of the intervention, all patients underwent a two-step hyperinsulinemic-euglycemic clamp (insulin infusion at 10 $mU \cdot m^{-2} \cdot min^{-1}$ and 40 $mU \cdot m^{-2} \cdot min^{-1}$, respectively) with primed D-[6,6-$^2H_2$]-glucose for 6 h (13) to assess hepatic and adipose tissue (10 $mU \cdot m^{-2} \cdot min^{-1}$) as well as peripheral (40 $mU \cdot m^{-2} \cdot min^{-1}$) insulin sensitivity, as described earlier (7), and an adipose tissue biopsy was collected after an overnight fast.

Exercise training protocol. Subjects participated in a 12-wk supervised, progressive exercise training program, as previously described (28). Aerobic exercise training was performed on a cycle ergometer twice a week for 30 min at 70% W_{max}, which was determined just before the start of the intervention. Resistance exercise training, which focused on large muscle groups (chest press, lat pull down, leg extension, shoulder press, horizontal row, leg press, triceps extensions, and biceps curls), was performed once a week and comprised 3 series of 10 repetitions at 60% of subjects' previously determined one repeated maximum (1RM). The 1RM test was preceded by a familiarization trial. Warming-up and cooling-down sessions of 5 min were performed on a stationary bike at 45% W_{max}. Every 4 wk, 1RM and $\dot{V}O_{2max}$ were reassessed, and training loads were adjusted accordingly to assure that the training stimulus was maintained. At baseline and after the 12-wk training program, several measurements were performed, as described below.

Adipose tissue biopsies. After an overnight fast, abdominal subcutaneous adipose tissue biopsies (~1 g) were collected 6–8 cm lateral from the umbilicus, under local anesthesia (2% lidocaine) by needle biopsy. To exclude any acute effects on gene and protein expression, postexercise training adipose tissue biopsies were collected 72 h after the last bout of exercise. The collected tissue was washed with sterile saline, and visible blood vessels were removed. One part of the biopsy was snap-frozen in liquid nitrogen and stored at −80°C for gene and protein expression analyses, whereas two parts were processed for determination of adipocyte morphology and measurement of ex vivo lipolysis, as described below.

Adipocyte morphology. A part of the adipose tissue biopsy was fixed overnight in 4% paraformaldehyde and embedded in paraffin for histological sections (8 μm). Sections were cut from paraffin-embedded tissue, mounted on microscope glass slides, and dried overnight in an incubator at 37°C. The sections were stained with hematoxylin (VWR, Radnor, PA) and eosin (Klinipath BV, Duiven, The Netherlands). Digital images were captured with a Leica DFC320 digital camera (Leica, Rijswijk, the Netherlands) at ×20 magnification (Leica DM3000 microscope, Leica), and computerized morphometric analysis (Leica QWin V3, Cambridge, UK) of individual adipocytes was performed in a blinded manner. Approximately 400 adipocytes per sample were measured.

Gene expression. Total RNA was extracted from frozen adipose tissue biopsies (~500 mg) using Trizol chloroform extraction (Invitrogen, Cergy Pontoise, France) and 300 ng RNA was reversed transcribed using iScript cDNA synthesis kit (BIO-RAD). Gene expression for markers of lipolysis [*ATGL* (*PNPLA2*), *HSL* (*LIPE*), *CGI-58*, and *PLIN1* (perilipin 1)], inflammation [*TNFα*, *IL-6*, *MCP-1* (*CCL2*), and *CD68*], browning (*CIDEA* and *PRDM16*), mitochondrial biogenesis [*PGC-1α* (*PPARGC1A*)], and adipokine expression (*ADIPOQ* and *LEP*) (Table 1 for primer sequences) was determined in a total volume of 25 μl containing 12.5 ng cDNA using SYBR Green-based quantitative PCR (iCycler/MyIQ, BIO-RAD). Results were calculated via the $2^{-\Delta CT}$ method and normalized for *18S* (housekeeping gene) ribosomal RNA.

Protein expression. First, subcutaneous adipose tissue (~500 mg) was ground to a fine powder under liquid nitrogen and homoge-

Table 1. *RT quantitative PCR primer sequences*

Genes	Sequences
ATGL	GTGTCAGACGGCGAGAATG
	TGGAGGGAGGGAGGGATG
HSL	GCGGATCACACAGAACCTGGAC
	AGCAGGCGGCTTACCCTCAC
CGI-58	CAGCATCCAGTCCTTACGACCA
	GTTCAGTCCACAGTGTCGCAGA
PLIN1	CTCTCGATACACCGTGCAGA
	TGGTCCTCATGATCCTCCTC
TNFα	CCGAGTGACAAGCCTGTAGC
	GAGGACCTGGGAGTAGATGAG
IL-6	AAATTCGGTACATCCTCGACGG
	GGAAGGTTCAGGTTGTTTTCTGC
MCP-1	CCCCAGTCACCTGCTGTTAT
	TCCTGAACCCACTTCTGCTT
CD68	CCCTATGGACACCTCAGCTTT
	GAAGGACACATTGTACTCCACC
CIDEA	TCAGACCTTGGGAGACAACACG
	CGAAGGTGACTCTCGCTATTCC
PRDM16	CAGCCAATCTCACCAGACACCT
	GTGGCACTTGAAAGGCTTCTCC
PGC-1α	TCTGAGTCTGTATGGAGTGACAT
	CCAAGTCGTTCACATCTAGTTCA
ADIPOQ	TGGTGAGAAGGGTGAGAA
	AGATCTTGGTAAAGCGAATG
LEP	GAACCCTGTGCGGATTCTTGT
	TCCATCTTGGATAAGGTCAGGAT

The following RT quantitative PCR primer sequences were used for gene expression analysis. Upper sequences represent forward primers (5′-3′), whereas lower sequences represent reverse primer (5′-3′).

nized in radioimmunoprecipitation assay buffer {10 mM Tris [Calbiochem]-HCl (Merck, Darmstadt, Germany)}, buffered saline (Merck) with 0.1% sodium dodecyl sulfate (Bio-Rad Laboratories Inc, Hercules, CA), 1% sodium deoxycholate (Sigma-Aldrich, St. Louis, MO), 1% NP-40 (Fluka), and a protease/phosphatase inhibitor cocktail (Cell Signaling Technology, Beverly, MA). The homogenate was lysed on iced, vortexed for 5 min, and centrifuged at 20,000 *g* for 30 min at 10°C. The infranatant was carefully collected, and aliquots were stored at −80°C. The protein concentration was determined by the Bradford-based protein assay (Santa Cruz Biotechnology, Dallas, TX).

Next, solubilized proteins (15 μg) were separated on a precast gel (Criterion TGX any kD, Bio-Rad Laboratories Inc) and transferred onto a nitrocellulose membrane (Trans Blot Turbo transfer system; Bio-Rad). Differences in loading were adjusted to total protein content [via Ponceau S (Sigma-Aldrich) staining], the same microgram of protein was loaded for each sample, and appropriate positive controls were included.

Thereafter, quantitative Western blot analysis was performed to determine the levels of the OXPHOS proteins. OXPHOS blots were probed with Total OXPHOS Antibody Cocktail (Mitoscience/Abcam, Cambridge, MA) and a secondary horseradish peroxidase-conjugated rabbit anti-mouse antibody (DakoCytomation, Glostrup, Denmark). Antigen-antibody complexes were visualized using chemiluminescence by a ChemiDoc XRS apparatus (Bio-Rad) and analyzed with Quantity One software (Bio-Rad), which calculated the optical density units that are expressed as average intensity (average intensity = total intensity of the rows of pixels inside the band boundary divided by the number of rows, minus the background intensity).

Ex vivo adipocyte lipolysis. Ex vivo adipocyte lipolysis was determined in a subgroup of 15 individuals with obesity because of the limited amount of available adipose tissue in 6 subjects. Mature adipocytes were isolated from the subcutaneous adipose tissue following collagenase digestion. First, digestion was performed for 60 min at 37°C in a Krebs-Ringer phosphate buffer containing 100 mg glucose/100 ml and 4% bovine serum albumin with 2 mg/ml collagenase (Sigma-Aldrich, Zwijndrecht, the Netherlands). Second, adipocytes (~5,000–10,000 cells/incubation) were incubated with or without increasing concentration of salbutamol (specific β_2-adrenergic receptor agonist) (10^{-9}–10^{-4} M; GlaxoSmithKline, Zeist, the Netherlands) for 2 h at 37°C in Krebs-Ringer phosphate buffer. Thereafter, incubation medium was collected and stored at −80°C until analysis. Glycerol concentration in the medium, which is an indicator of complete triacylglycerol (TAG) hydrolysis (lipolysis), was determined using the EAPL-200 EnzyChrom Adipolysis Assay Kit (Glentaur Europe BVBA, Kampenhout, Belgium).

Biochemistry. Arterialized blood samples were collected and immediately centrifuged at 4°C for 10 min at 1,000 *g*, and plasma was snap-frozen in liquid nitrogen and stored at −80°C until further analysis. Plasma nonesterified fatty acid (NEFA) (Wako NEFA C test kit; Wako Chemicals, Neuss, Germany) and glucose (hexokinase method; LaRoche, Basel, Switzerland) concentrations were measured with enzymatic assays, whereas TAG concentrations were measured colorimetrically (Roche, Vienna, Austria), automated on a Cobas Fara/Mira. Plasma insulin and serum liver function parameters (aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transpeptidase) were routinely measured and analyzed at the clinical chemistry department in the hospital.

Statistics. Student's paired *t*-test was used to determine the effects of the exercise training program. All variables were checked for normal distribution and were natural log-transformed to satisfy conditions of normality (PLIN, IL-6, Leptin, PGC-1α expression). All data are presented as means ± SE. Calculations were done using SPSS 21 for Mac OS X (IBM, Chicago, IL). $P < 0.05$ was considered statistically significant.

RESULTS

Anthropometric and clinical characteristics. Subject characteristics before and after the 12-wk supervised, progressive exercise training program are summarized in Table 2. As expected, $\dot{V}O_{2max}$ ($P < 0.001$), W_{max} ($P < 0.001$), and 1RM ($P < 0.001$) were significantly increased following the exercise

Table 2. *Anthropometric and clinical subject characteristics before and after the 12-wk supervised, progressive exercise training program*

	Baseline	Postintervention	*P* Value
Age, yr	58.1 ± 1.6		
Body weight, kg	95.4 ± 2.6	95.4 ± 2.6	0.963
BMI, kg/m^2	30.0 ± 0.6	30.0 ± 0.6	0.998
Fat mass, kg	27.9 ± 1.2	27.2 ± 1.3	0.021
Fat-free mass, kg	65.7 ± 1.5	66.0 ± 1.6	0.315
Body fat percentage, %	28.8 ± 0.7	28.2 ± 0.7	0.004
$\dot{V}O_{2max}$, ml·min^{-1}·kg^{-1}	26.9 ± 0.9	29.5 ± 1.0	<0.001
W_{max}, W/kg	2.0 ± 0.8	2.4 ± 1.0	<0.001
Strength, kg	83.4 ± 3.8	97.1 ± 4.7	<0.001
Fasting plasma glucose, mmol/l	6.6 ± 0.5	6.6 ± 0.5	0.949
Fasting insulin, mU/l	13.1 ± 1.5	12.4 ± 1.4	0.262
HOMA-IR	3.7 ± 0.5	3.4 ± 0.4	0.304
Fasting plasma NEFA, μmol/l	702 ± 31.7	648 ± 38.8	0.131
Fasting plasma TAG, mmol/l	1.6 ± 0.1	1.6 ± 0.2	0.790
R_d, μmol·kg^{-1}·min^{-1}	11.5 ± 2.2	14.2 ± 2.8	0.047
EGP suppression, %	−48.6 ± 4.5	−53.4 ± 5.6	0.226
NEFA suppression, %	−61.6 ± 3.2	−62.1 ± 3.2	0.864

Data are expressed as mean ± SE (n = 21 participants). BMI, body mass index; EGP, endogenous glucose production (EGP suppression reflects hepatic insulin sensitivity); NEFA, nonesterified fatty acid (NEFA suppression reflects adipose tissue insulin sensitivity; R_d, glucose rate of disappearance (reflects peripheral insulin sensitivity); TAG, triacylglycerol; $\dot{V}O_{2max}$, maximal aerobic capacity; W_{max}, maximal power output.

training intervention. The training program significantly increased peripheral insulin sensitivity ($P = 0.047$), whereas hepatic ($P = 0.226$) and adipose tissue insulin sensitivity ($P = 0.864$) were not significantly affected (Table 2). Furthermore, total fat mass ($P = 0.021$) and body fat percentage ($P = 0.004$) were slightly but significantly decreased, whereas body weight ($P = 0.963$), body mass index ($P = 0.998$), fat free mass ($P = 0.315$), and plasma glucose, NEFA, and TAG concentrations remained unaltered after the training intervention (Table 2).

Adipocyte morphology. Representative images of adipose tissue sections, stained with hematoxylin-eosin, are depicted in Fig. 1*A*. The exercise training intervention did neither affect mean adipocyte size (pre: 62.9 ± 1.4 vs. post: 63.3 ± 1.4 μm, $P = 0.744$, Fig. 1*B*) nor adipocyte size distribution (Fig. 1*C*).

Gene expression. The exercise training did not alter adipose tissue gene expression of *ATGL* ($P = 0.253$, Fig. 2*A*), *HSL* ($P = 0.875$, Fig. 2*B*), *PLIN1* ($P = 0.733$, Fig. 2*C*), or *CGI-58* ($P = 0.966$, Fig. 2*D*). Furthermore, the inflammatory markers *TNFα* ($P = 0.858$, Fig. 2*E*), *IL-6* ($P = 0.353$, Fig. 2*F*), *MCP-1* ($P = 0.144$, Fig. 2*G*), and *CD68* ($P = 0.686$, Fig. 2*H*) were unchanged after the intervention. Next, gene expression of the browning markers *CIDEA* ($P = 0.901$, Fig. 2*I*) and *PRDM16* ($P = 0.872$, Fig. 2*J*) and *PGC-1α* ($P = 0.793$, Fig. 2*K*), a major regulator of mitochondrial biogenesis and function, remained unchanged following the exercise training. Finally, gene expression of *ADIPOQ* ($P = 0.340$, Fig. 2*L*) and *LEP* ($P = 0.951$, Fig. 2*M*) was also not significantly altered after the 12-wk training program. Of note, adipose tissue UCP-1 mRNA was not detectable (data not shown).

Protein expression. Total OXPHOS protein expression remained unchanged following the exercise training program (pre: 27.0 ± 9.7 vs. post: 24.9 ± 6.9 AU, $P = 0.789$, Fig. 3*A*). More specifically, OXPHOS complex I (pre: 2.9 ± 1.2 vs. post: 2.9 ± 1.0 AU, $P = 0.989$, Fig. 3*B*), complex II (pre: 9.2 ± 2.4 vs. post: 8.9 ± 1.7 AU, $P = 0.850$, Fig. 3*C*), complex III (pre: 4.7 ± 2.7 vs. post: 2.1 ± 1.0 AU, $P = 0.330$, Fig. 3*D*), complex IV (pre: 0.8 ± 0.2 vs. post: 0.9 ± 0.3 AU, $P = 0.780$, Fig. 3*E*), and complex V (pre: 14.5 ± 6.8 vs. post: 13.3 ± 5.3 AU, $P = 0.819$, Fig. 3*F*) were not affected.

Ex vivo adipocyte lipolysis. The potency of salbutamol to stimulate lipolysis was determined by its EC_{50}, which represents the concentration of agonist inducing 50% of its maximal lipolytic response, in a subgroup of individuals ($n = 15$) (Table 3). Importantly, comparable effects of the training program on anthropometric and clinical parameters were found in this subgroup of individuals in comparison with the total study population. The dose-response curve for salbutamol on ex vivo adipocyte lipolysis is presented in Fig. 4. The exercise training intervention did not induce a significant change in basal lipolysis (pre: 7.0 ± 0.9 vs. post: 5.9 ± 0.6 μmol glycerol·10^7 cells^{-1}·2 h incubation^{-1}, $P = 0.108$), maximal lipolysis (pre: 18.1 ± 2.1 vs. post: 14.5 ± 1.9 μmol glycerol·10^7 cells^{-1}·2 h incubation^{-1}, $P = 0.111$), or the potency of salbutamol ($-\log EC_{50}$) to stimulate lipolysis (pre: 6.0 ± 0.2 vs. post: 5.8 ± 0.3, $P = 0.555$).

DISCUSSION

The aim of the present study was to investigate the effects of a 12-wk supervised, progressive exercise training program on the abdominal subcutaneous adipose tissue phenotype in metabolically well-phenotyped individuals with obesity. Here, we

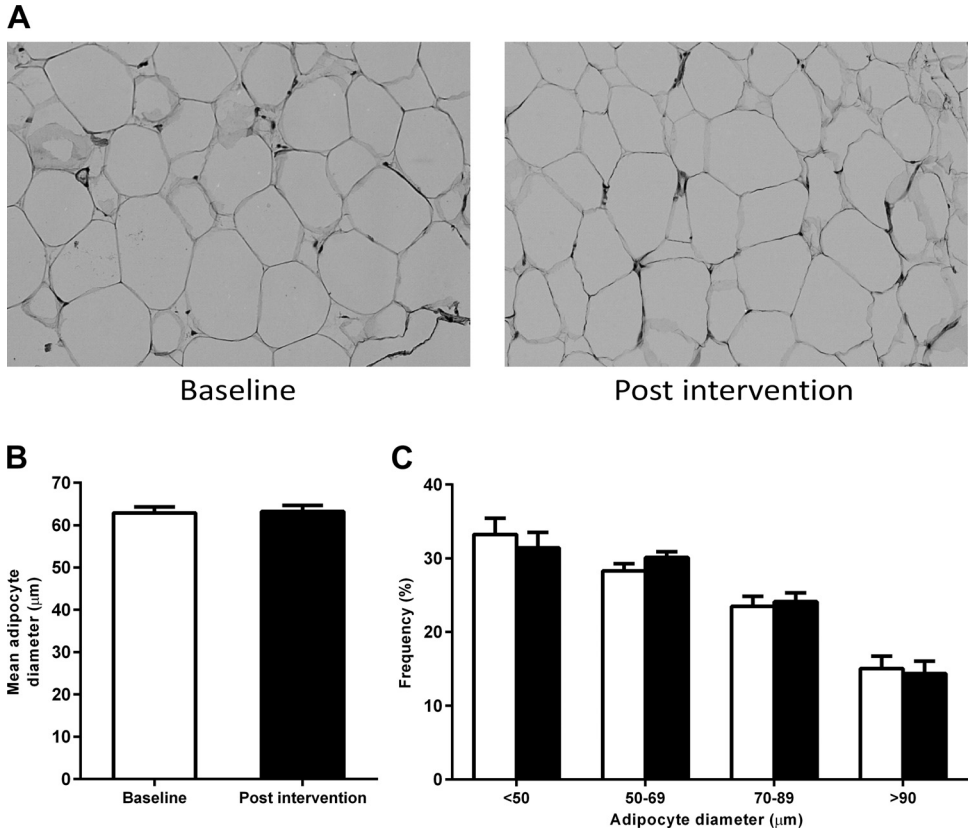

Fig. 1. Exercise training-induced effects on adipose tissue morphology. Representative images of adipose tissue sections stained with hematoxylin-eosin (×20 magnification) (*A*). Mean adipocyte size (*B*) and adipocyte size distribution (*C*) are presented as mean ± SE ($n = 21$). Data were analyzed using a Student's paired *t*-test. Open bars indicate baseline values, and closed bars indicate postintervention values (*B* and *C*).

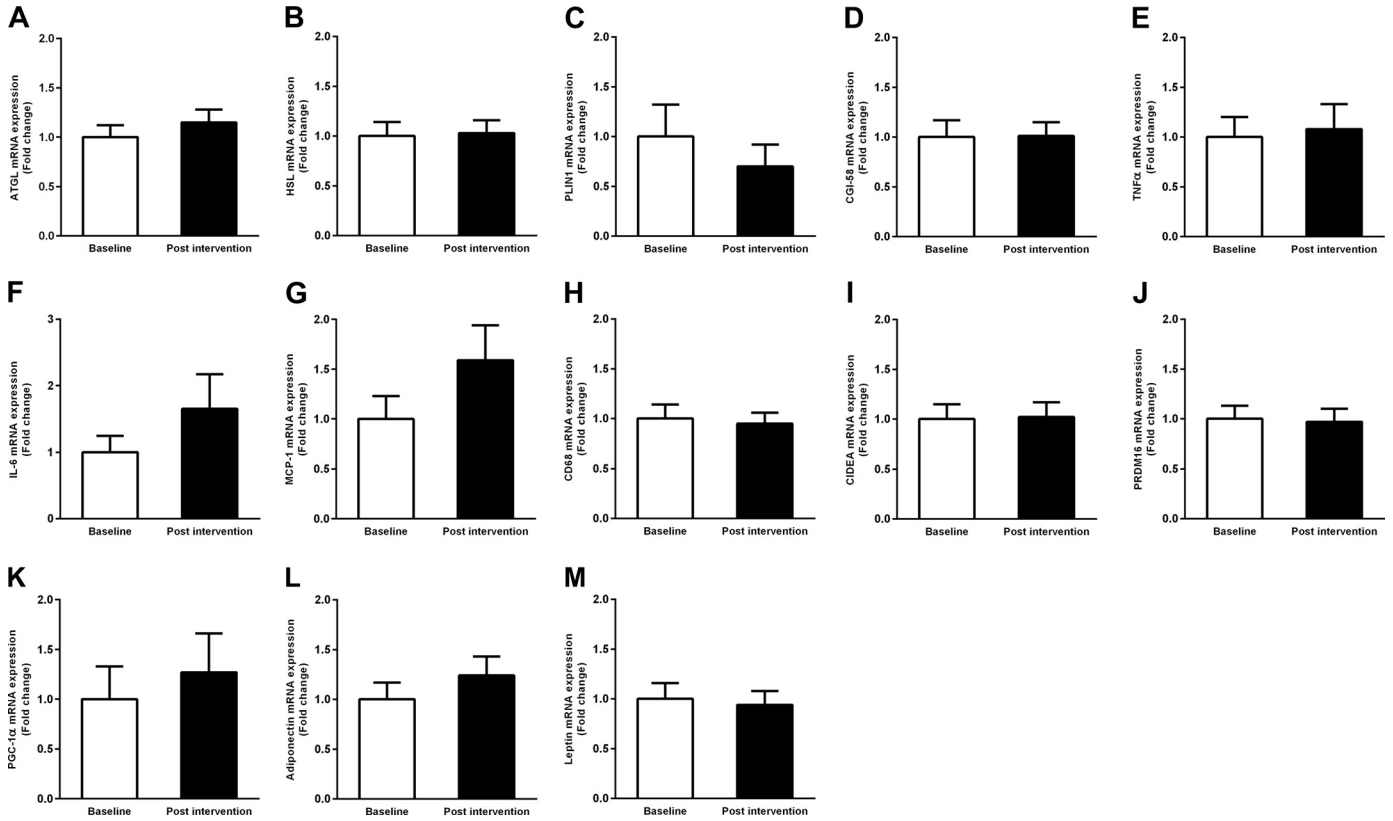

Fig. 2. Exercise training-induced effects on adipose tissue gene expression. Gene expression of markers of lipolysis (*A–D*), inflammation (*E–H*), browning (*I* and *J*), mitochondrial biogenesis and function (*K*), and adipokines (*L* and *M*). Data are presented as mean fold-change (± SE) relative to the baseline values (n = 21) and were analyzed using a Student's paired *t*-test. Open bars indicate baseline values; closed bars indicate postintervention values.

demonstrate that exercise training did neither alter abdominal subcutaneous adipocyte morphology and adipose tissue gene expression of markers for mitochondrial biogenesis/function, browning, lipolysis, inflammation and adipokines, adipose tissue OXPHOS protein content, nor β_2-adrenergic stimulation of adipocyte lipolysis in subjects with obesity. These data suggest that alterations in the phenotype of abdominal subcutaneous adipose tissue do not significantly contribute to the exercise-induced improvement in peripheral insulin sensitivity in men with obesity when adipose tissue mass is only slightly reduced (~0.7 kg).

The training program induced a significant increase in aerobic capacity, maximal power output, and maximal muscle strength, indicating that the supervised, progressive nature of the program was successful regarding the enhancement of physical fitness. This was accompanied by a slight but signif-

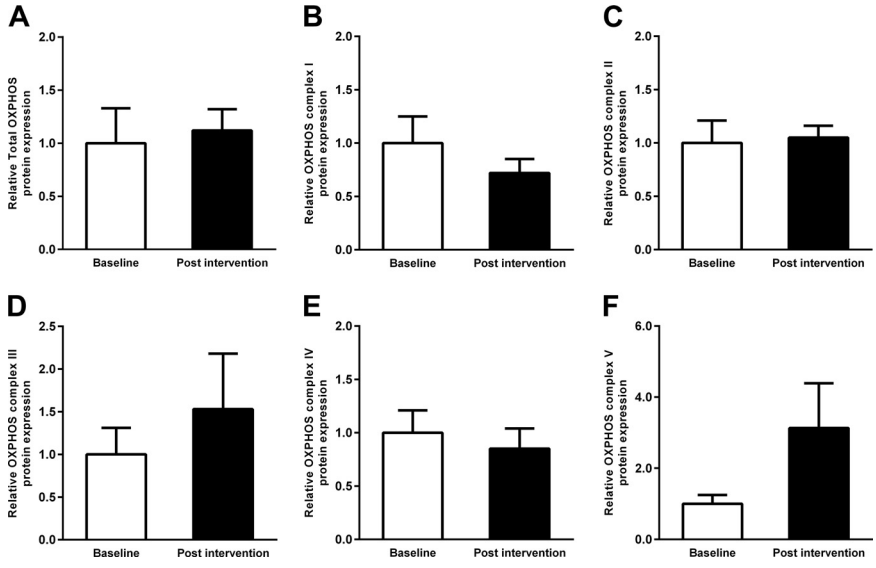

Fig. 3. Exercise training-induced effects on adipose tissue mitochondrial oxidative phosphorylation (OXPHOS) protein expression. Protein content of total OXPHOS (*A*), OXPHOS complex I (*B*), OXPHOS complex II (*C*), OXPHOS complex III (*D*), OXPHOS complex IV (*E*), and OXPHOS complex V (*F*). Data are expressed as mean fold-change (± SE) relative to baseline values (n = 12) and were analyzed using a Student's paired *t*-test. Open bars indicate baseline values; closed bars indicate postintervention values.

Table 3. *Anthropometric and clinical characteristics of the subgroup of individuals in which ex vivo adipocyte lipolysis was determined before and after the 12-wk supervised, progressive exercise training program*

	Baseline	Postintervention	*P* Value
Age, yr	55.8 ± 2.2		
Body weight, kg	97.1 ± 2.9	96.9 ± 3.1	0.777
BMI, kg/m^2	30.1 ± 0.6	30.1 ± 0.7	0.704
Fat mass, kg	29.5 ± 1.4	28.6 ± 1.5	0.027
Fat free mass, kg	65.4 ± 1.7	65.7 ± 1.9	0.378
Body fat percentage, %	30.1 ± 0.0	29.3 ± 0.0	0.004
$\dot{V}O_{2max}$, ml·min^{-1}·kg^{-1}	26.8 ± 1.1	29.5 ± 1.3	0.001
W_{max}, W/kg	2.0 ± 0.1	2.4 ± 0.1	<0.001
Strength, kg	79.7 ± 4.8	96.7 ± 5.3	<0.001
Fasting plasma glucose, mmol/l	6.6 ± 0.6	6.4 ± 0.6	0.561
Fasting insulin, mU/l	14.9 ± 2.2	12.8 ± 1.9	0.548
HOMA-IR	4.3 ± 0.7	3.9 ± 0.8	0.914
Fasting plasma NEFA, μmol/l	682 ± 33.0	650 ± 33.9	0.244
R_d, μmol·kg^{-1}·min^{-1}	11.7 ± 2.4	13.5 ± 4.4	0.355
EGP suppression, %	−44.1 ± 4.8	−44.6 ± 6.6	0.481
NEFA suppression, %	−60.8 ± 4.0	−64.1 ± 3.2	0.462

Data are expressed as mean ± SE (n = 15). BMI, body mass index; EGP, endogenous glucose production (EGP suppression reflects hepatic insulin sensitivity); NEFA, nonesterified fatty acid (NEFA suppression reflects adipose tissue insulin sensitivity); R_d, glucose rate of disappearance (reflects peripheral insulin sensitivity); $\dot{V}O_{2max}$, maximal aerobic capacity; W_{max}, maximal power output.

icant decrease in fat mass and body fat percentage. As observed earlier (7), peripheral insulin sensitivity was significantly increased, whereas hepatic and adipose tissue insulin sensitivity remained unchanged after the training program.

Exercise training interventions may affect adipocyte morphology in humans (49). We demonstrated that a 12-wk exercise training intervention did not significantly alter mean adipocyte size or adipocyte size distribution, despite a 0.7-kg decrease in total fat mass. In contrast, Despres et al. (14) demonstrated that 20 wk of endurance training decreased mean adipocyte size in young men but not in women. Importantly, in the latter study, a more pronounced reduction in body weight (~3.0 kg) was found. Thus, a more prolonged or a more intense exercise training intervention, leading to a more pronounced decrease in adipose tissue mass, seems necessary to induce beneficial changes in adipocyte morphology.

Since an altered rate of lipolysis is one of the characteristics of adipose tissue dysfunction and relates to peripheral insulin resistance (47), we determined adipose tissue gene expression of lipolytic enzymes and genes encoding lipid droplet-associated proteins. Conflicting results on the impact of exercise training on basal and stimulated adipose tissue lipolysis in humans has been reported, as extensively reviewed (49). Here, we show that exercise training did not alter the expression of genes related to lipolysis under fasting conditions in individuals with obesity. It has previously been demonstrated that β_2-adrenergic stimulation of lipolysis is impaired in obese as compared with lean subjects, whereas β_1-adrenergic receptor sensitivity was comparable between groups (40). We therefore determined if exercise training altered β_2-adrenergic sensitivity of lipolysis. In agreement with unchanged adipose tissue gene expression of lipolytic markers, the potency of the β_2-adrenergic receptor agonist salbutamol to stimulate abdominal subcutaneous adipocyte lipolysis was comparable before and after the exercise training program. Furthermore, basal and maximal β_2-adrenergic receptor-mediated lipolysis remained unchanged after exercise training. The present findings are in agreement with a previous study showing no improvement of β_2-adrenergic stimulation of lipolysis after 12 wk of training in nondiabetic men with obesity (12). However, in contrast to the present findings, a decreased basal lipolysis was found after exercise training in the latter study (12). This may be explained by the modest loss of fat mass in the present study, which did not result in a reduction in adipocyte size. Indeed, it has been previously demonstrated that substantial weight loss, which decreased adipocyte size, increased and normalized the sensitivity to catecholamine-stimulated lipolysis in subjects with obesity (34).

In addition to impairments in lipolysis, a proinflammatory phenotype of adipose tissue is associated with insulin resistance in obese subjects and patients with T2DM (16, 38, 47). In the present study, no changes in macrophage infiltration and inflammatory markers in adipose tissue were found following exercise training. The absence of alterations in the inflammatory profile after exercise training is in agreement with most previous studies in subjects with obesity (22, 32, 42, 50). Furthermore, we found no effects of exercise training on adipose tissue gene expression of leptin and adiponectin, which is in accordance with observations in both lean and overweight/obese humans (2, 22, 32). Nevertheless, some studies have shown a reduction in adipose tissue gene expression of *MCP-1* (42) and an increase in adiponectin expression (50) following exercise training in subjects with obesity. The present findings further support the notion that a reduction in adipocyte size seems required to achieve beneficial changes in the adipose tissue phenotype.

Rodent studies demonstrated that increasing brown adipose tissue mass/activity or inducing browning of white adipose tissue via cold exposure or other stimuli might be a promising strategy for the treatment of obesity and obesity-related impairments in glucose homeostasis (18). Interestingly, physical exercise has been shown to induce browning of white adipose tissue in rodents, possibly via the secretion of the myokine irisin (5), although findings are controversial (15), or via increased natriuretic peptide concentrations (4). In the present

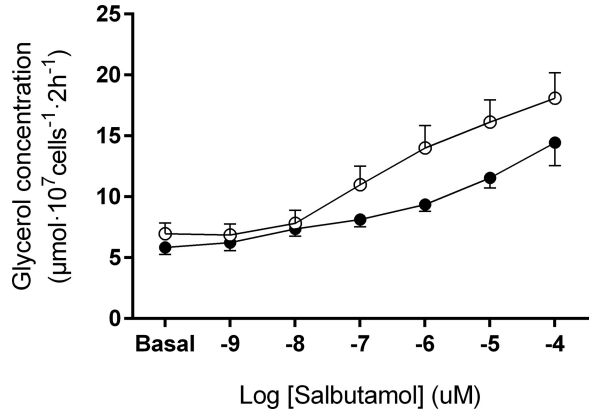

Fig. 4. The effects of salbutamol on ex vivo adipocyte lipolysis before and after exercise training. Glycerol release into the medium (μmol glycerol·10^7 cells^{-1}·2 h incubation^{-1}) was used as an indicator of lipolysis. Data are presented as mean ± SE (n = 15) and were analyzed using a Student's paired t-test. Open circles represent baseline values; closed circles represent postintervention values.

study, the expression of *PRDM16* and *CIDEA*, markers of beiging/browning of white adipose tissue, did not change following the intervention. In agreement with our findings, no exercise training-induced changes in browning markers in white adipose tissue were found in lean (8, 31), overweight (31, 37), or obese (52) subjects. In line, the expression of browning markers in white adipose tissue of endurance-trained athletes was not different from lean sedentary controls (54). Thus, while exercise seems to induce a brown phenotype in rodent adipose tissue, there is no clear evidence thus far that exercise training elicits beiging of white adipose tissue in humans, as extensively reviewed very recently (1, 26).

The oxidative phenotype of white adipose tissue is impaired in obesity and seems related to an altered glucose homeostasis in rodents and humans (19, 24, 41, 55). Here, we found that the exercise training did not induce alterations in adipose tissue gene expression of *PGC-1α*, which is in accordance with most (2, 8, 37), but not all, previous studies (21, 39). Furthermore, we observed no significant exercise-induced alterations in adipose tissue OXPHOS protein content after the training intervention, which is in line with previous reports on high-intensity interval training in healthy lean (8) and overweight (25) subjects. In contrast to findings in humans, previous rodent studies have shown that endurance training has beneficial effects on intraabdominal and epididymal white adipose tissue mitochondrial activity (44), indicating species differences in mitochondrial protein expression and/or function in response to exercise.

A limitation of the present study is that we did not include a control group (no exercise). Importantly, however, several previous randomized, placebo-controlled studies in overweight and obese humans have demonstrated that adipocyte size and adipose tissue gene expression were not significantly altered following placebo treatment for 2–6 mo (17, 30, 33). Therefore, it is unlikely that the negative findings with respect to exercise-induced effects on adipocyte morphology and function in the present study are due to the lack of a control group. Moreover, the volume of exercise performed by the study participants was relatively low, yet significant improvements in maximal aerobic capacity and power output, as well as peripheral insulin sensitivity, were found. Future studies should examine the effects of other exercise regimens, varying in duration, intensity, and/or type of exercise, on adipose tissue function and related metabolic health. Also, potential exercise-induced changes in other fat depots (e.g., visceral or gluteofemoral adipose tissue) remain to be investigated.

In conclusion, the present study demonstrated that a 12-wk supervised, progressive exercise training intervention, which improved physical fitness and peripheral insulin sensitivity, had neither effects on abdominal subcutaneous adipocyte morphology, adipose tissue gene and protein expression of markers related to adipose tissue function, nor ex vivo β_2-adrenergic sensitivity in male subjects with obesity. Noteworthy, we cannot exclude that exercise training may induce beneficial alterations in the adipose tissue phenotype after a more prolonged or more intense intervention, leading to a more pronounced loss of fat mass, or may induce changes in other fat depots (i.e., visceral or gluteofemoral fat).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

P.S. and G.H.G. conceived and designed research; R.S., B.B., and K.F.T.-B. performed experiments; R.S., B.B., and K.F.T.-B. analyzed data; R.S., B.B., J.W.J., E.E.B., K.F.T.-B., M.K.H., M.A.v.B., P.S., and G.H.G. interpreted results of experiments; R.S. prepared figures; R.S. drafted manuscript; R.S., B.B., J.W.J., E.E.B., K.F.T.-B., M.K.H., M.A.v.B., P.S., and G.H.G. edited and revised manuscript; R.S., B.B., J.W.J., E.E.B., K.F.T.-B., M.K.H., M.A.v.B., P.S., and G.H.G. approved final version of manuscript.

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